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Evidence for a Polyalkyl Nature of Soil Humin

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Most of the studies on the soil organic matter have been carried out on colloidal substances soluble in alkaline extractants (humic acids and fulvic acids). The other major fraction – the humin – represents about half of the stabilized, biologically resistant pool of organic carbon on the Earth's surface [1]. Since their resistance to biodegradation, plant lignins have frequently been considered as major precursors of humin [1 – 3].

The classical concepts of the nature of the humin have been questioned over the last few years. Recent studies using ^{13}C NMR spectrometry point to the need of identifying the nature of paraffinic material in the fine soil fractions [4]. The origin of the polymethylene structures in soil humus remains particularly obscure since the lipids removed by solvent extraction always represent a minimal amount to the ^{13}C NMR alkyl signals. The possibility of the accumulation in soils of highly aliphatic kerogen-like materials constitutes a suggestive hypothesis that would connect the early humification stages with the formation processes of fossil organic sediments of economic interest [5].

The soils studied here (Al horizons) were representative of the three types of terrestrial humus of different biological activity: a *mull* from a Typic Rendoll under oak, a *moder* from an Entic Luvisol under beech, and a *mor* from an Entic Haplorthod under a heather [6–8].

The soil fractions of undecomposed plant particles, free lipids, strongly sequestrated organic matter, and the free and clay-associated humic and fulvic acids were sequentially removed by standard procedures [2, 9, 10]. The final residue – humin fraction – amounted to 21–36 % of the soil C.

These soil residues were subjected to seven repeated treatments with 5 % $NaBO_2 \cdot H_2O_2$ at 90 °C (ca. 80 % degradation). In addition to high yields of alkali-soluble macromolecular fractions (recovery ca. 50 %), such a method [7] released diagnostic molecules (ca. 4 %) where other drastic degradation procedures yielded unspecific products [10]. Although the hydrogen peroxide is known to open some aromatic rings, yielding short-chain alkyl products [11], the oxidation of the humic substances with reagents such as $KMnO_4$ or H_2O_2 under drastic conditions [12] produces resistant benzenecarboxylic and phenolic acids; the selective preservation of these compounds during degradation has been considered to cause an overestimation of the aromaticity of the humus substances [13]. The macromolecular fractions (50–56 %C, 5–6 %H, 34–39 %O and 4–6 %N) were recovered from the digest by precipitation with HCl, and purified by methods similar to those used for the soil humic acids [7]. Their CP/MAS ^{13}C NMR spectra [14] revealed that most of the carbon was in paraffinic structures (0–46 ppm; Fig. 1); minor peaks were for N- and O-alkyl carbons (46–110 ppm) and aromatic carbons (110–160 ppm). Evidence for additional amounts of lignin-like structures [15], protein, and fungal perylenequinonic pigments [8, 16] was obtained from the infrared and visible derivatographic spectroscopic patterns (6*N* HCl hydrolysis yielded amino acids of between 10–16 %).

The low-molecular-weight products were recovered from the acidified digest by repeated extractions with ethyl acetate at room temperature, methylated with ethereal diazomethane, and silylated with trimethylsilylimidazole. By using gas chromatography-mass spectrometry we identified up to 150 compounds (Table 1). Only traces of alkanes and low proportions of fatty acids were found: the bulk of the depolymerization products were di- or polyfunctional compounds. The wide (C_3–C_{22}) series of α,ω-dibasic acids (bell-shaped with a maximum at C_9) were predominant, and there were substantial amounts of ω-OH fatty acids, di-, tri-, tetra-, and penta-polyhydroxymonobasic acids, coinciding with the monomers proposed for the plant lipid polyesters [17]. The most frequent aromatic compounds were phenolic acids. Such degradation patterns resembled those we obtained after perborate depolymerization of cork samples from *Quercus suber* L. but differed from those obtained from humic acids, where aromatic and fatty acids were the most frequent degradation products [7]. The present results conform to those found with BF_3-MeOH transesterification, a specific method to remove ester links, although in that case a considerable amount of the humin remained after depolymerization [6]. Such resistant material may be the origin of a portion of the above-mentioned α,ω-diacid series, the chain-length distribution of which suggests polyalkyl structures derived from 8-, 9-, or 10-unsaturated lipids, not necessarily inherited from plant biopolyesters. The tendency of unsaturated fatty acids to form polymers by autoxidation and photooxygenation is well known [18]. We have observed that the hexane-insoluble rubbers formed by 48-h air-drying of UV-irradiated linseed oil at room temperature also yielded – upon perborate depolymerization – a series of α,ω-diacids similar to those obtained from the soil fractions under study.

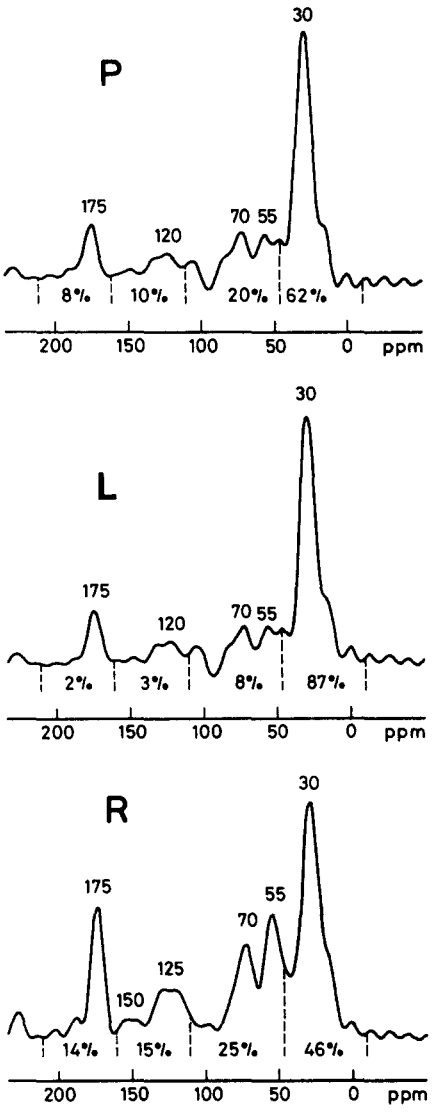

Fig. 1. CP/MAS ^{13}C NMR spectra of acid-insoluble colloids released by sodium perborate depolymerization from humin from three forest soils (Podzol, Luvisol, Rendoll). Quantitative values are shown on the corresponding spectral regions

In conclusion, our results show that the humins had macromolecular polymethylene structures which could be derived from:

(1) The accumulation of higher-plant hydroxyfatty acid polymers such as cutins and suberins. Their intrinsic resistance to biodegradation may be enhanced by the sterical protection of some of the ester bonds [19] after microbial reworking and association with clay.

(2) The probable formation in soils of cross-linked structures from polyunsaturated lipids in part derived from microbial metabolism. Such mechanisms have been considered to play a key role in the synthesis of marine humic substances from fatty acids of planktonic organisms [20], and we have observed that similar reactions are encouraged in soil microaggregates by higher oxygenation and substrate concentration, as well as the presence of mineral catalysts.

Table 1. Summary of organic compounds[a] released after sodium perborate depolymerization of soil humins

Compound type	C range	Major compounds	Number of compounds	Humus type[b] *mull*	*moder*	*mor*
Monobasic acids	$C_{10}-C_{20}$	Methyl hexadecanoate Methyl *iso*-heptadecanoate Methyl octadecanoate	15	6.4	3.1	0.1
Dibasic acids	C_3-C_{20}	Dimethyl α,ω-octanedioate Dimethyl α,ω-nonanedioate Dimethyl α,ω-decanedioate	30	39.1	51.1	24.9
β-Hydroxymonobasic acids	C_8-C_{14}	Methyl β-hydroxy *iso*-decanoate	5	0.9	0.2	0.4
ω-Hydroxymonobasic acids	$C_{14}-C_{18}$	Methyl ω-hydroxyhexadecanoate Methyl ω-hydroxyoctadecanoate	5	2.2	1.4	2.2
Dihydroxymonobasic acids	C_{16}	Methyl 8,16-dihydroxyhexadecanoate Methyl 9,16-dihydroxyhexadecanoate Methyl 10,16-dihydroxyhexadecanoate	3	6.0	4.5	13.5
Hydroxydibasic acids	$C_{14}-C_{16}$	Methyl 8-hydroxy hexadecane-1,16-dioate Methyl 9-hydroxy hexadecane-1,16-dioate Methyl 10-hydroxy hexadecane-1,16-dioate	7	3.1	4.0	12.2
Trihydroxymonobasic acids	C_{18}	Methyl *erythro*-9,10,16-trihydroxyoctadecanoate Methyl *threo*-9,10,18-trihydroxyoctadecanoate	2	1.3	2.2	3.0
Pentahydroxymonobasic acid	C_{18}	Methyl 9,10,12,13,18-pentahydroxyoctadecanoate	1	0.7	0.8	1.1
Unidentified aliphatics			12	16.3	13.9	10.6
Phenolic acids		Methoxy benzenecarboxylic acid methyl ester Dimethoxy benzenecarboxylic acid methyl ester Trimethoxy benezenecarboxylic acid methyl ester Methoxy benzenedicarboxylic acid dimethyl ester Methoxy benzenetricarboxylic acid trimethyl ester	17	10.7	7.7	18.7
Benzenecarboxylic acids		1,2-Benzenedicarboxylic acid dimethyl ester 1,2,4-Benzenetricarboxylic acid trimethyl ester 1,2,4,5-Benzenetetracarboxylic acid tetramethyl ester Methyl benzenecarboxylic acid methyl ester	11	8.9	8.4	10.5
Miscellaneous		Methyl *p*-methoxy cinnamate Methyl 3,4-dimethoxy cinnamate Furandicarboxylic acid dimethyl ester Propanetricarboxylic acid trimethyl ester	2 2			

[a]As methyl esters.
[b]Percentages from the peak areas in the gas chromatograms (flame ionization detector). Peaks representing less than 0.1 % were not accounted for.

(3) The contribution of very resistant nonsaponifiable lipid polymers, the presence of which in higher plants has been postulated recently [21].

Although only direct evidence of hypothesis (1) can be taken into account after comparing the present results with those available for the known biogenic lipid polymers, the contribution of additional resistant structures is required to explain that only a portion of humin is readily depolymerized upon repeated transesterifications.

In any case, no evidence for the predominance of the alkyl structures found in humin was obtained when soil humic acids were examined by chemical degradation or ^{13}C NMR spectrometry [22], suggesting that humin should not be considered a priori as the final product of insolubilization in soil of humic colloids.

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